

Claims

1. A complex formed by a hybridisation reaction comprising four nucleic acid molecules; the complex comprising a target nucleic acid molecule and first, second and third nucleic acid probe molecules; wherein the first probe comprises a foot region which is complementary to a first portion of the target and is hybridised thereto, and an arm region which is substantially non-complementary to the target; the second probe comprises a foot region which is complementary to a second portion of the target, such that the foot region of the second probe is hybridised to the target adjacent or substantially adjacent to the foot region of the first probe, the second probe also comprising an arm region which is substantially non-complementary to the target but which is complementary and hybridised to the arm region of the first probe; the third probe being complementary, at least in part, to a portion of the arm region of the first probe, such that the third probe is hybridised to the arm region of the first probe adjacent or substantially adjacent to the second probe; and wherein formation of the complex creates a functional double-stranded RNA polymerase promoter, one strand of the promoter being provided by the first probe, and the other strand being provided jointly by the second probe and by the third probe.
2. A complex according to claim 1, wherein at least one of the first, second or third probes comprises PNA and/or LNA.
3. A complex according to claim 2, wherein the first and/or second probe comprises PNA and/or LNA.
4. A complex according to any one of claims 1, 2 or 3, comprising a functional double stranded T3, T7 or SP6 RNA polymerase promoter.

5. A complex according to any one of the preceding claims, comprising single or double stranded sequence adjacent to the promoter which increases the activity of the promoter.
6. A complex according to claim 5, wherein one of said probes comprises a +12 sequence.
7. A complex according to claim 5, wherein the first probe comprises a +12 sequence.
8. A complex according to any one of the preceding claims, comprising a sequence which, when transcribed into RNA, facilitates isolation, identification, detection, quantification or amplification of the transcript.
9. A complex according to any one of the preceding claims, wherein one of said probes comprises a destabilizing moiety.
10. A complex according to any one of the preceding claims, wherein the second and third probes form a discontinuous sequence of an RNA polymerase promoter template strand.
11. A complex according to any one of claims 1-9, wherein the second and third probes form a discontinuous sequence of an RNA polymerase promoter non-template strand.
12. A method of detecting the presence of a target nucleic acid molecule in a sample, the method comprising the steps of: contacting the sample comprising the target with first and second nucleic acid probes, each probe comprising a foot region complementary to respective first and second portions of the target, which portions are adjacent or substantially so; wherein the first and second probes each further comprise an arm region substantially non-complementary to the target, at least part of the arm region of

the first probe being complementary to at least part of the arm region of the second probe, such that respective foot regions of the first and second probes hybridise to the target, allowing hybridisation of the complementary parts of the arm regions of the first and second probes; and causing to be present a third nucleic acid probe molecule which is complementary to a portion of the arm region of the first probe, such that the third probe hybridises to the first probe adjacent or substantially adjacent to the arm region of the second probe, thereby creating a functional double-stranded RNA polymerase promoter, one strand of the promoter being provided by the first probe, the other strand being provided jointly by the second and third probes; causing RNA synthesis from the RNA promoter; and detecting, directly or indirectly, the RNA so synthesised.

13. A method according to claim 12, performance of which results in the formation of a complex in accordance with any one of claims 1-11.
14. A method according to claim 12 or 13, wherein RNA produced from the functional RNA promoter is amplified prior to detection.
15. A method according to any one of claims 12, 13 or 14, wherein RNA produced from the functional RNA promoter is detected directly or indirectly via a method which involves use of a molecular beacon or fluorophore.

A complex comprising three nucleic acid molecules: a target nucleic acid sequence; a first probe; and a second probe; wherein the first probe comprises, in the 5' to 3' direction, a template portion transcribable by an RNA polymerase, and a template strand of an RNA polymerase promoter, a target complementary portion which is hybridised to at least a 3' end region of the target sequence; and wherein the second probe is hybridised to the first probe adjacent or substantially adjacent to the 3' end of the target sequence, the second probe comprising part of the non-template strand complementary to the template strand of the promoter present in the first probe, the remaining part of the non-template strand of the promoter sequence being present at the 3' end of the target sequence; the arrangement being such that formation of the complex creates a functional double stranded RNA polymerase promoter, with a discontinuity in the non-template strand, between the second probe and the target sequence.

16. A method of detecting in a sample the presence of a nucleic acid target sequence; the method comprising the steps of: contacting a first and second probe as defined above, with the sample, so as to form the complex of claim 16; and detecting directly or indirectly RNA transcripts of the template portion of the first probe.